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TITLE: A Chemopreventive Trial to Study the Effects of High Tea Consumption on Smoking-Related Oxidative Stress

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13. ABSTRACT (Maximum 200 Words)
Our overall goal is to develop a sage and feasible model for the chemopreventive of a wide range of tobacco-related diseases. Our immediate goal, that is addressed over a 4-year study period, is to determine the effects of high tea consumption on biological markers of oxidative stress that mediate lung cancer risk. We are conducting a 6-month randomized, controlled, double-blinded chemopreventive trial in a group of COPD subjects who are being randomized to green or black tea preparations or a control intervention (matching placebo). Levels of 8-hydroxydeoxyguanosine and 8-F2-isoprostanes will be use dot measure DNA and lipid damage respectively. Changes in biomarkers of oxidative damage will be measured in urine, blood, and exhaled breath condensate. The study protocol was approved by all parties in September 2003. Recruitment and screening of participants for eligibility criteria started in October 2003. By the end of December, 28 participants signed the consent form and were screened for eligibility criteria (spirometry for lung function). Eight subjects with FEV1>85% of the standard were excluded for the study and 3 subjects dropped out. Currently 17 eligible subjects were enrolled in the study. Eleven subjects are completing the 1-month run-in period and 8 subjects have been randomized. We expect that adherence to a regular pattern of tea is feasible and quantifiable among this high risk population.

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INTRODUCTION

Preventive strategies require identification of cancer-susceptible individuals resulting from combinations of carcinogen exposure and lack of protective factors. Oxidative reactions have been implicated as important modulators of human health and can play a role in both disease prevention and disease development. A large number of studies have demonstrated an increased oxidant burden and consequently increased markers of oxidative stress in the airspaces, breath, blood, and urine of smokers and of patients with chronic obstructive pulmonary disease (COPD) [1,2]. Changes in dietary habits with the intake of more cancer-chemopreventive agents appear to be a practical approach for cancer prevention in subjects with increased oxidative stress as is the case of subjects with COPD and ≥ 25 pack/year of smoking history. The present study will investigate the ability of regular green and /or black tea consumption to decrease oxidative stress during the context of a randomized, controlled, double blinded, dietary intervention trial. Levels of 8-hydroxydeoxyguanosine (8-OHdG) will be used to measure DNA damage and levels of 8-F2 isoprostanes (8-epi-PGF2) and ethanes will be used to measure lipid damage. Testing for biomarkers of oxidative stress in exhaled breath condensate (EBC) will complement other innovative methods currently being investigated. The use of this novel strategy might enable further classification of people at risk of increased oxidative stress lung cancer, such as smokers, workers in nuclear weapons plants, Gulf War veterans [3], and US Marines by degree of risk. Such refinement of risk analysis might then be used to identify candidates for screening studies.

BODY

Task 1. <u>Preparation, protocol development and analysis of tea extracts and placebo</u> (QC/QA) for tea polyphenols (Months 1-7)

a) <u>All interviewers will be trained in the specific protocols and administration of questionnaires</u>.
All the interviewers were trained in the specific protocols and administration of study questionnaires.

b) <u>Obtain Human subjects approval</u>
A detailed study protocol was developed, revised and approved by both USAMRAA and the University of Arizona 's human Subject committees. Consent and HIPPA forms were developed and approved by both USAMRAA and the University of Arizona 's human Subject committees. Final approval obtained on September 30, 2003. Advertisement, screening, and recruitment for the study started in October 2003.

c) <u>Preparation of recruitment materials (brochures, Advertisements.)</u>
Immediately after obtaining final approval study, advertisement of the study started and brochures were distributed to COPD clinics. The study cups (12 oz cups with study logo) , timers (3 minutes timer with study logo and clinic phone number), and bags (tote bags with study logo to carry forms and sealed urinary cups) were ordered and received.

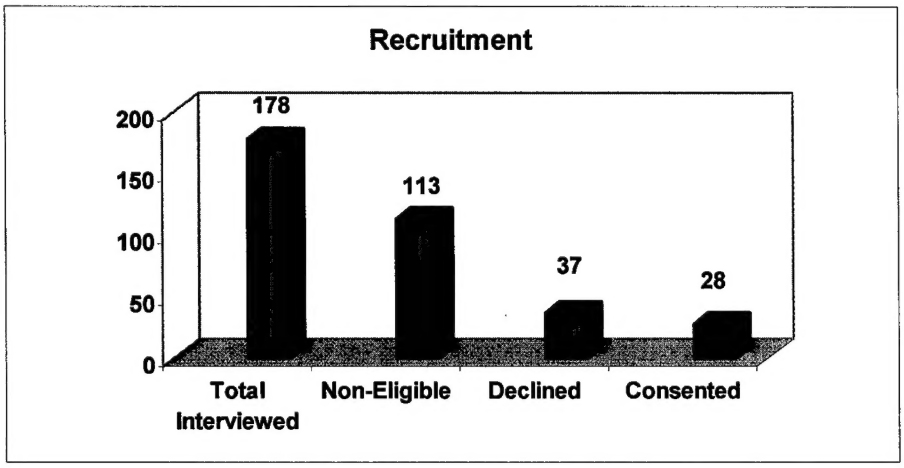
d) <u>Obtain the study green tea , black tea and matching placebo.</u>
The study agents (green tea, black tea, and matching placebo) were ordered and received in July. All the tea bags have blank labels and were received in large barrels labeled as A, B, and C. The code is kept in a sealed envelop to be used by the medical director if needed (as in a health related emergency). Randomization is done separately under the direction of Dr. Harris the

Epidemiologist and all Subjects' tea packages are sent un-identified (only with subject ID) to the study clinic to ensure complete blindness of both staff and subjects.

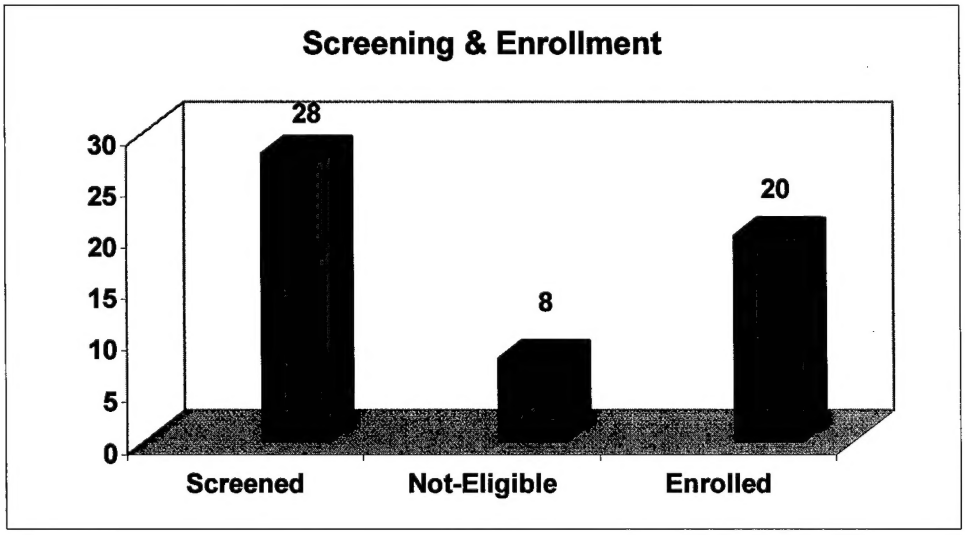
Task 2. **Recruitment/ eligibility, Run-In & baseline assessment of oxidative stress (Month 8-36)**

a & b) Potentially eligible subjects will be recruited beginning in month 5 of the study and continuing through the end of year 3 and complete baseline questionnaires.

A total of 178 subjects were interviewed by phone for eligibility criteria. One hundred and thirteen subjects were not eligible because of age, pack/year of cigarettes, medications, had cancer, or currently enrolled in another study. Thirty-seven subjects refused to participate (won't give up tea, cannot drink much tea, study too long).



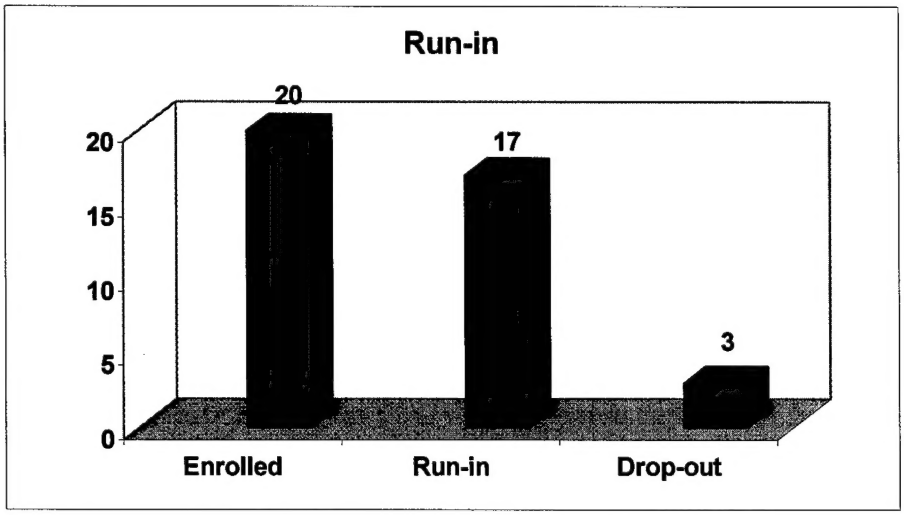
By the end of December (2003), 28 participants signed the consent form and were screened for confirmation of COPD eligibility criteria (spirometry for lung function tests). Eight subjects with FEV1 > 85% of the standard were excluded from the study and the remaining 20 eligible (12 females and 8 males) subjects were enrolled in the study.

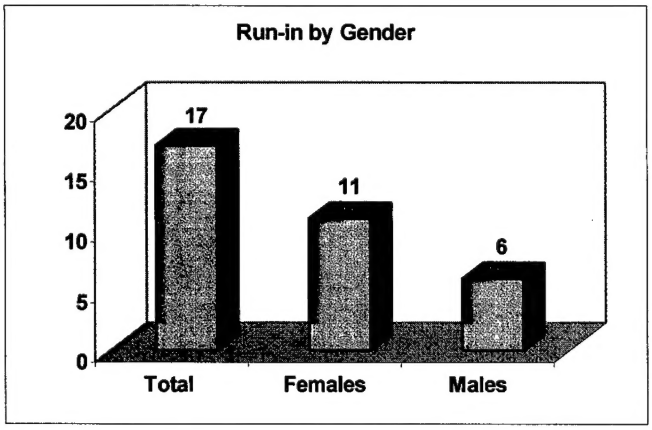


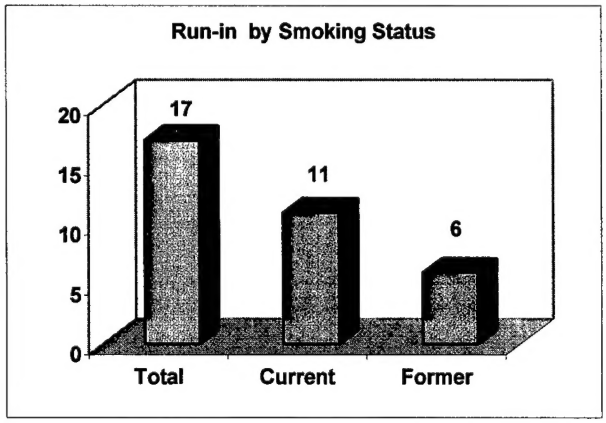
c) Eligible subjects will complete 1-month run-in period during which they will consume the placebo beverage and complete all baseline questionnaires.

By the end of December (2003), twenty eligible subjects completed all baseline questionnaires and started the run-in period. Each enrolled participant, received 1-month of placebo tea bags, study teacup, a 3-minute timer, the monthly diary and health monitoring forms, and sterile urine cups. Subjects were contacted biweekly to ensure and encourage adherence and to monitor any adverse event.

Three of the participants dropped-out from the study in the first week Reported causes of drop-out are : 1) could not stop coffee, diagnosed with prostate cancer, and caffeine intolerance. To date, 17 participants have completed and/or are completing the 1-month run-in.







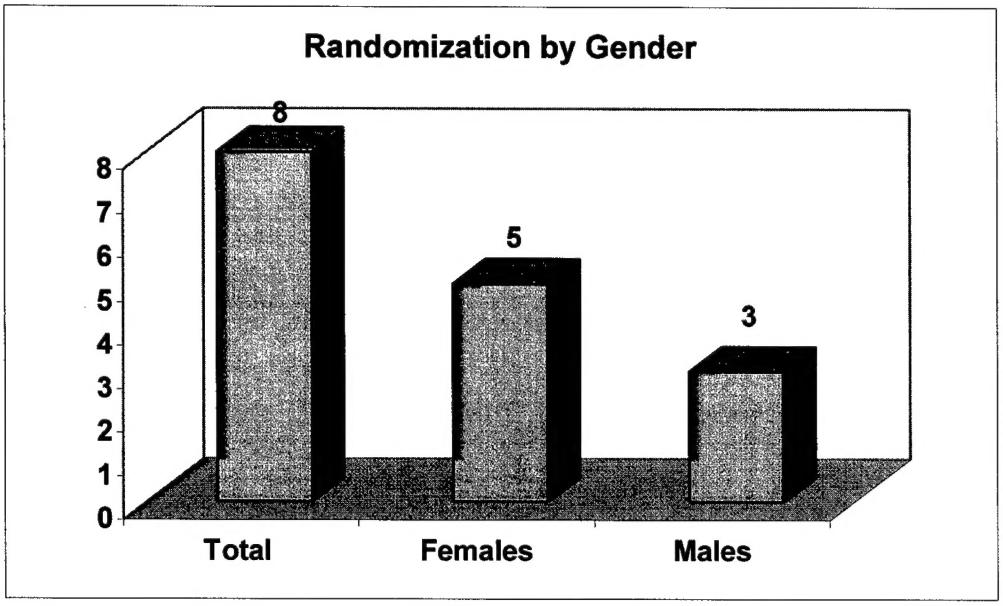
d & e) Subjects who complete the run-in period will provide blood, urine and exhaled breath condensate (EBC) samples for biomarker analysis. Subjects will be asked to provide buccal cells and induced sputum samples for storage.

To-date 8 subjects completed the run-in successfully and were randomized to 1 of the study arms: Green tea, black tea, or placebo. All randomized subjects provided blood and urine samples, exhaled breath condensate (EBC), and buccal cell samples. Seven of the randomized subjects provided sputum samples. The rest of subjects (n=9) are completing the run-in period without ant reported adverse event or drop-out.

Task 3. **Intervention, Follow-up & Exit focus groups to study the effect of tea consumption on DNA (8-OHdG) and lipid (8-epi-PGF2) damage in blood, urine, and EBC (Months 10-43).**

a) Randomize eligible COPD chronic and former smokers into one of three interventions: black tea, green tea or placebo for 6 months.

To-date 8 subjects have been randomized to 1 of the 3 arms of the study. The rest of the participants (n=9) will be randomized upon successfully completing the run-in period.



b) To maintain high adherence to the study intervention including collection of blood, urinary, and EBC samples through the 6-month intervention period and 1-month follow-up period.

Study participants are contacted biweekly by phone to ensure adherence. Subjects complete a tea and smoking diary in which they report their daily intake of tea (amount and time) and the number of cigarettes smoked each day. They also complete a health monitoring form in which they report any change in medication use , any health-related event ,or any perceived adverse event.

Task 4. **Laboratory analyses and data entry (Months 8-45)**

a) Quality control assurances of laboratory methods

We have completed all the validation and quality control measures for the biomarkers of oxidative stress. Our quality control and validation data show that the urinary biomarkers of oxidative DNA and lipid damage are stable even when left at room temperature for 3 consecutive days.

KEY RESEARCH ACCOMPLISHMENTS

- Development and approval of the study protocol
- Development and approval of all study forms and questionnaires
- Successful recruitment and screening
- Successful enrollment in the study
- Successful collection of biological samples (blood, urine, EBC, buccal and sputum samples)
- Validation and quality control of all laboratory methods

REPORTABLE OUTCOMES

An abstract was submitted to the "Peer Reviewed Medical Research Program Investigators Meeting" that will be held in Puerto Rico, April 26-28, 2004.

CONCLUSIONS

Although recruitment started in October 2003, we were able to reach a large number of potential participants. We interviewed (initial screening) 178 subjects and enrolled 20 eligible subjects in the study. Interviewing and initial screening is ongoing and we plan to enroll and randomize at least 50 participants this year.

Because tea is one of the most popular beverages consumed worldwide, the relationship between tea consumption and human cancer incidence is an important concern. Tea can be easily consumed with one's ordinary meals making compliance and adherence to dietary intervention more likely to succeed. Thus, the role of tea drinking as a potential inhibitor of carcinogenesis merits careful evaluation. We believe that a program of nutritional intervention with realistic dietary modifications that are effective, safe, and acceptable should be the cornerstone of any cancer prevention strategy.

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APPENDICES

1. Abstract entitled" A Chemoprevention Trial To Study The Effects Of High Tea Consumption On Smoking-Related Oxidative Stress"

A CHEMOPREVENTION TRIAL TO STUDY THE EFFECTS OF HIGH TEA CONSUMPTION ON SMOKING-RELATED OXIDATIVE STRESS

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BACKGROUND/PURPOSE: Oxidative reactions have been implicated as important modulators of human health and can play a role in both disease prevention and disease development. A large number of studies have demonstrated an increased oxidant burden and consequently increased markers of oxidative stress in the airspaces, breath, blood, and urine of smokers and of patients with chronic obstructive pulmonary disease (COPD). The overall goal of this study is to develop a safe and feasible clinical research approach that will serve as a model for the chemoprevention of a wide range of tobacco-related diseases. Our immediate goal, that is addressed over a 4-year study period, is to determine the effects of high tea consumption on biological markers of oxidative stress that mediate lung cancer risk, including, 8-hydroxydeoxyguanosine (8-OhDG), F2-isoprostanes (8-epi-PGF2), ethanes, and nitric oxide. **METHODS**: We are conducting a 6-month randomized, controlled, double-blinded chemopreventive trial in a group of COPD subjects (FEV1 $\leq$ 85% of the standard) with 25 or more pack-years of smoking history. The participants are stratified on smoking status (current or former) and gender, and are being randomized to green or black tea preparations or a control intervention (matching placebo). Levels of 8-OHdG will be used to measure DNA damage and levels of 8-epi-PGF2 and ethanes will be used to measure lipid damage. Changes in biomarkers of oxidative damage will be measured in urine, blood and exhaled breath condensate. **RESULTS:** The study protocol was approved by all parties in September 2003. Recruitment and screening of participants for eligibility criteria started in October 2003. By the end of November, 20 participants (13 females and 7 males) signed the consent form and were screened for eligibility criteria (spirometry for lung function tests). Seven subjects with FEV1 > 85% of the standard were excluded from the study and the remaining 13 eligible subjects were enrolled in the study and are completing the 1-month run-in phase. **CONCLUSION**: We expect that adherence to a regular pattern of tea is feasible and quantifiable among this high risk population.